248-Pos

A Functional, Pentameric Form of Phospholamban Is Required For Two-Dimensional Crystallization With the Sarcoplasmic Reticulum Calcium Pump

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A Functional, Pentameric Form Of Phospholamban Is Required For Two-Dimensional Crystallization With The Sarcoplasmic Reticulum Calcium Pump
Phospholamban physically interacts with the sarcoplasmic reticulum calcium pump (also known as SERCA) and regulates contractility of the heart in response to adrenergic stimuli. We have studied this interaction using electron microscopy of large two-dimensional crystals of SERCA in complex with phospholamban. In our original work, phospholamban oligomers were found interspersed between dimer arrays of SERCA and a three-dimensional model was constructed to show potential interactions between the two proteins. In the present study, we have examined the effects of phospholamban phosphorylation and mutation on the formation of two-dimensional co-crystals with SERCA. Phospholamban phosphorylation at Ser^{16} and a well-characterized loss-of-function mutation (Asn^{34}-to-Ala) significantly reduced crystal formation. More importantly, projection maps calculated from these crystals revealed that the densities attributable to phospholamban become disordered, suggesting a reduced interaction with SERCA. In contrast, a pentameric gain-of-function mutant (Lys^{27}-to-Ala) significantly enhanced crystal formation. These latter crystals were used to calculate an improved projection map from frozen-hydrated crystals to a resolution of 8 Å. We conclude that the oligomeric state of phospholamban in the crystals is a pentamer, and that phosphorylation and mutation of phospholamban alter physical interactions in the crystals in a manner that is consistent with a functional association with SERCA. Combined, the data suggest that the pentameric state of phospholamban is not simply an inactive storage form.

249-Pos

Towards the Development of Rationally Designed Phospholamban Mutants For Treatment of Heart Failure

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PLN is the endogenous inhibitor of the sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA), the integral membrane enzyme responsible for 70% of the Ca2+ shuttling into the SR, inducing cardiac muscle relaxation in humans. Dysfunctions in SERCA:PLN interactions have been implicated as having a critical role in cardiac disease. Inspired by the success of S16E, a pseudo-phosphorylated form of PLN which successfully reduced the progression of the cardiac failure in murine models and large animals upon delivery via rAAV gene therapy, I wish to further develop paradigms to rationally design therapeutic mutations based on the foundation of biophysical data known about the system, thereby ''tuning'' phospholamban structural dynamics to directly affect SERCA function. The present study utilizes a combination of NMR spectroscopy and coupled-enzyme assays to investigate the functional contribution of the structural dynamics of both loss-of-function (LOF) and naturally occurring malignant mutants of phospholamban, and draw correlations between the structural dynamics of the inhibitor to the activity of its target enzyme. These studies further develop the model by which the control of enzyme function is performed by altering the structural dynamics of a small inhibitor can then be translated to other membrane enzymes, such as the Na/K-ATPase.

250-Pos

Phospholamban Topology As a Regulator of Sarcoplasmic Reticulum Ca2+ATPase Function

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Sarcoplasmic reticulum Ca^{2+}ATPase (SERCA) transports Ca^{2+} ions from the cytosol of cardiomyocytes into the SR, making it a crucial regulator of cardiac muscle function. SERCA is inhibited by phospholamban (PLN), a membrane-spanning protein consisting of a transmembrane (TM) helix connected to an amphipathic helix by a short loop. Phosphorylation of PLN at Ser16 relieves the inhibition of SERCA but does not dissociate the PLN-SERCA complex. Owing to their pivotal role in cardiac muscle function, SERCA and PLN have received much attention as therapeutic targets. In one such study, a pseudo-phosphorylated S16E mutant of PLN was successfully used to relieve the effects of heart failure in hamsters (*Nat. Med*., 2002, 8, 864-871). Ongoing efforts in our lab are aimed to design new PLN mutants that bind tightly with SERCA, but relieve inhibition (i.e., mimic Ser16 phosphorylation).
A recent study refined the structure and topology of PLN in a lipid bilayer environment using a hybrid solution and solid state NMR approach (***PNAS***, 2009, 106, 25, 10165-101670). The tilt angle of the PLN TM helix in DOPC/DOPE bilayers was determined to be 24o with respect to the bilayer normal. In this study, we reconstituted PLN into magnetically aligned bicelles and used PISEMA experiments to show that the topology of PLN is strongly dependent on hydrophobic mismatch, since mutations in the PLN sequence and changes in the bilayer composition alter the TM helix tilt angle. Taken with results from our lab that correlated PLN structural dynamics to inhibitory function on SERCA (***J. Biol. Chem***., 2007, 282, 51, 37205-14), the differences in topology presented in this work introduce a new dimension in the study of PLN mutants that could be crucial for understanding the complex structural features of PLN that are important for SERCA regulation.

251-Pos

Solid State NMR Observation of the Geometry of Kinked Protein Helices

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Oriented sample solid state nuclear magnetic resonance (ssNMR) is a technique for characterizing the structure of membrane proteins in a nearly native environment. 15N anisotropic chemical shift and 15N-1H dipolar couplings in two dimensional separated local field ssNMR spectra provide precise restraints on the orientation of peptide planes with respect to the membrane normal in alpha helical protein structures. These restraints can be utilized in structure determination and provide a straightforward technique for characterization of helical orientation using PISA wheel analysis. PISA wheel analysis provides the tilt angle and rotational orientation of helical structures. This analysis breaks down for helices with a kink, because the tilt angles to the membrane normal can be determined but the 3D geometry of the kink and how it relates to the membrane environment is not known from the PISA wheel analysis.
Here, we present the detailed mathematical analysis of the geometry of the kink and how it relates to the ssNMR spectra. This analysis will be applied to the M2 proton channel from the influenza A virus. The kinked helical system will be modeled as two idealized helices with a single pair of modified phi/psi angles. The kink angle is calculated as a function of the modified phi/psi angles. The PISA wheel analysis yields the rotation and tilt angles for each idealized section of helix. These values are used to derive a relationship between the tilt and rotation angles and the kink phi/psi angles. The result links the ssNMR data to the structure of a kinked helix. Specifically important to the structure of membrane proteins is the known relationship of the structure to the membrane normal. This information can then be used in structural refinement using ssNMR and other structural data.

252-Pos

Structural and Functional Studies of M2 Proton Channel From Influenza A Virus

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M2 protein of influenza A virus forms a homo-tetrameric proton channel involved in modifying virion and trans-Golgi pH during virus infection and inhibited by drugs Amantadine and Rimantadine. Previously determined structures for the membrane domain of M2 protein in detergent micelles have shown different conformations of TM helices with respect to channel axis i.e. tilt as well as relative orientation than structures solved in lipid bilayer environment. We report a new three dimensional structure of closed channel state of M2 protein residue (22-62) in native like PC:PE bilayer that encompasses all functionally relevant domains for proton channel activity.
In order to obtain high resolution structure in bilayer environment, M2(22-62) was expressed and purified from E. Coli. membrane and reconstituted in liposomes. Liposomal assays demonstrated fully active and amantadine-sensitive channels with an average proton uptake of 21.1 ± 1.9 H+/tetramer/s. Multidimensional Solid State NMR experiments performed on uniform 15N labeled and amino acid specific labeled M2(22-62) reconstituted in a DOPC:DOPE(4:1) lipid bilayer generated precise orientational restraints for amide bond vectors and peptide planes for each residue to determine three-dimensional structure. Helical tilt and rotation were calculated using dipolar couplings, chemical shift wave and polarization inversion slant angle (PISA) wheel analysis. Tetramer assembly and membrane equilibration was performed using molecular dynamics simulations. Structure shows four-helix bundle with TM and amphipathic helices tilted at ~34 degrees and ~105 degrees to channel axis, respectively with a tight turn joining two helices. Although the oligomeric state of the channel is stabilized due to the interactions of amphipathic helices as previously reported, membrane interaction and rotational orientation of amphipathic